with steroids. Example 14 provides studies of the anti-inflammatory properties of the TwHF extract. A new method for separating and quantitating triptolide and tripdiolide is provided in Example 15 and Example 16 describes the effect of TwHF extract on progesterone metabolism, highlighting the utility of the present invention for reducing progesterone, and the various physiological conditions that are related to progesterone (i.e., pregnancy). Example 17 provides therapeutic preparations of the TwHF extract. Example 18 provides methods for screening for agents having GR binding activiy and a steroid-sparing effect. Example 19 relates to the treatment of rheumatoid arthritis (RA) with an extract of *Tripteyglum wilfordii Hook F*. Example 20 relates to preparation of *tripteryglum wilfordii Hook F* composition. Example 21 relates to *Tripteyglum wilfordii Hook F* preparation and joint inflammation. Example 22 relates to the fact that treatment wth an ethyl acetate extract of *Tripteryglum wolfordii Hook F* improves joint imflammation in HLA B27-transgenic rats.

Example 1
EFFECT OF T_2 ON HUMAN LYMPHOCYTE FUNCTION

This example describes the effect of the *T. wilfordii* preparation, T_2, on *in vitro* immune responsiveness of human peripheral blood mononuclear cells (PBMC) obtained from normal individuals. It was found that the preparation exerted a concentration-dependent profile of suppressive activity on both T cell and B cell functions, whereas the functional activities of monocytes were more resistant to the suppressive effects of this *T. wilfordii* preparation with chloroform/methanol.

Methods

Cell preparation. PBMC were obtained from the blood of healthy adults by centrifugation on sodium diatrizoate/Ficoll gradients (Sigma, St. Louis, MO). Monocytes were isolated from PBMC by centrifugation on Sepra-cell-MN (Sepratech, Oklahoma City, OK) or by glass adherence. The monocytes obtained from the two procedures were used to examine interleukin-1 (IL-1) production and antigen presentation, respectively. For purification of T cells and B cells, PBMC were

incubated with L-leucine methyl ester HCl (Sigma) for 45 minutes at room temperature to deplete monocytes and natural killer cells[51]. The resultant lymphocytes were rosetted with neuraminidase-treated sheep red blood cells (SRBC) and were then separated by Ficoll/diatrizoate centrifugation[52]. T cells were further purified by passage of the rosette-positive population over a nylon-wool column to remove residual B cells and monocytes[53]. B cells were prepared from the initial population of rosette-negative cells by removing any remaining cells that formed rosettes with neuraminidase-treated SRBC.

Staining with monoclonal antibodies (MAb) to CD3 and CD20 and analysis with the fluorescence-activated cell sorter (FACS) indicated that the T cell and B cell populations were more than 96% and 90% pure, respectively. T cells were incubated with mitomycin c (0.1 mg/ml) for 45 minutes and then washed thoroughly[54].

Reagents, The *T. wilfordii* preparation, T_2, used in these studies was a chloroform/methanol extract prepared from the woody portion of the roots of TWH obtained from Taizhou Pharmaceutical Company (Taizhou, Jiang Su, People's Republic of China). This preparation, T_2, contained more than 8 different compounds including glycosides, diterpenoids, alkaloids, and ketones. Before use, the extract was dissolved in DMSO and further diluted with culture medium. Phytohemagglutinin (PHA; Wellcome Reagents, Research Triangle Park, NC), phorbol dibutyrate (PDB; Sigma), ionomycin (Calbiochem, San Diego, CA), and the anti-CD3 MAb, 64.1, were used for T cell activation[55]. MAb 64.1 was purified as previously described [Hansen *et al.*, "T cell protocol", *Leukocyte Typing*. Edited by Bernard, *et al.* Berlin, Springer-Verlag, 1982]. Human recombinant interleukin-2 (rIL-2; Cetus, Emeryville, CA) and/or formalinized *Staphylococcus aureus* (SA; Calbiochem) was used for B cell activation. The MAb against the α chain of the IL-2 receptor (IL-2R), anti-Tac, was obtained from Dr. Thomas Waldmann (NIH, Bethesda, MD) and was used to analyze IL-2R expression. Interleukin-1 (Cistron Technology, Pine Brook, NJ) was purchased for standardization of the IL-1 assay. Affinity-purified goat anti-human IgA, IgG, and IgM and similar antibodies conjugated to horseradish peroxidase were purchased from

Tago (Burlingame, CA). Streptokinase (SK) and tetanus toxoid (TT) were purchased from Hoechst-Roussel (Somerville, NJ) and MCDC Biologics (Jamaica Plain, MA), respectively.

Cell culture and assay of lymphocyte DNA synthesis. T cells (1 x 10^5/well) or B cells (5 x 10^4/well) alone or B cells with mitomycin c-treated T cells (1 x 10^5/well) were cultured in RPMI 1640 medium (Hazleton Biologics, Lenexa, KS) supplemented with 10% fetal calf serum, penicillin G (200 units/ml), gentamicin (10 µg/ml), and L-glutamine (0.3 mg/ml) in 96-well microtiter plates in a total volume of 200 µl, with or without the stimuli indicated, and in the presence or absence of various concentrations of T_2. The final concentration of DMSO in culture was 0.02-0.002%. This concentration of DMSO had no effect on any of the responses analyzed.

For both T and B cell activation, immobilized anti-CD3 (MAb 64.1) stimulation was used. This MAb was immobilized by incubating 50 µl (5 µg/ml) in each well for at least 2 hours at room temperature. The excess soluble antibody was removed before cell culture (Hansen, supra). Cells were cultured for the indicated duration, and then pulsed with 1 µCi of ^{3}H-thymidine, (^{3}H-TdR; New England Nuclear, Boston, MA) for the last 12 and 18 hours for T cell and B cell cultures, respectively. ^{3}H-TdR uptake was measured in a liquid scintillation counter. All data are expressed as the mean counts per minute of 3 replicate determinations[56].

IL-1 production assay. Monocytes (1 x 10^5/well) were suspended in RPMI 1640 medium with 1% normal human serum (NHS) and cultured with or without lipopolysaccharide (10 µg/ml) in the presence or absence of various concentrations of T_2 for 24 hours. The culture supernatants were collected, and serial dilutions were assayed for IL-1 using C3H/HeJ murine thymocytes as described elsewhere[57]. Concentrations of T_2 contained in the dilutions of supernatants had no effect on DNA synthesis by C3H/HeJ thymocytes.

IL-2 production assay. T cells (1 x 10^5/well) were incubated with or without PHA (1 μg/ml) or immobilized anti-CD3 in the presence or absence of various concentrations of T_2 for 24 hours. Cell-free supernatants were harvested, serial dilutions were made, and IL-2 content was assayed with CTLL-2 cells as described previously[58].

IL-2R expression. T cells were cultured with or without the indicated stimuli in the presence or absence of various concentrations of T_2 for 36 hours. After washing, the cells were stained with saturating concentrations of anti-Tac or a mouse IgG control MAb, followed by fluorescein isothiocyanate-conjugated goat anti-mouse Ig antibody (Cappel, West Chester, PA). The samples were fixed with 1% paraformaldehyde and analyzed with a FACSTAR (Becton Dickinson, Mountain View, CA) flow cytometer, using a single-histogram statistics program (Davis, supra).

Measurement of Ig synthesis. The amount of IgG, IgA, and IgM in the culture supernatants of B cells stimulated with SA plus rIL-2 in the presence or absence of T_2 for 7 days was determined using an isotype-specific enzyme-linked immunosorbent assay method. Quantitation of the Ig in the supernatants was then determined by comparison with a standard curve. The sensitivity of the assay is 15 ng/ml for IgA and IgG, and 30 ng/ml for IgM[59].

Results

Effect of T_2 on human T cell responsiveness. These studies demonstrate that T_2 caused concentration dependent inhibition of PHA induced ^{3}H-thymidine incorporation by purified human T lymphocytes (FIG. 1). Fifty percent inhibition was noted at concentrations of approximately 0.2 μg per ml. Cell cycle analysis indicated that T_2 prevented cells from progressing through the G1 phase of the cell cycle (FIG. 2A-2L). Mitogen induced IL-2 production by purified T-cells was also inhibited by a similar concentration of T_2 (FIG. 3). Mitogen induced expression of IL-2 receptors was not inhibited by T_2 (Table 1) indicating that it was nontoxic to this cellular activity. These results suggested that the decrease in proliferation might be the result of inhibition of IL-2 production.